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Functional Characterization of DNA Repair Proteins

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Purpose: Genetic material (DNA) is susceptible to spontaneous decomposition, to attack by reactive chemicals produced naturally in cells, and to assault by environmental and food mutagens. DNA modifications can lead to permanent genetic changes that promote human disease. To combat the deleterious effects of DNA damage, organisms are equipped with DNA repair systems. The focus of our investigations has been to elucidate the details of mammalian DNA repair. These studies have provided important insights into the relationship of DNA repair to human disease and the genetic factors that contribute to individual susceptibility to the harmful effects of environmental mutagens (e.g. ionizing radiation), and have established a framework for designing more effective anti-cancer treatment schemes.

Approach: To investigate questions related to DNA repair, its mechanism(s) and linkage to disease development, we are employing an array of molecular, cellular, biochemical and structural approaches. These complementary experimental techniques have permitted a detailed analysis into various processes of human DNA repair.

Technical Accomplishments: Sites of base loss (or AP sites) are frequently formed DNA lesions corrected by a multistep pathway initiated by an AP endonuclease. In mammals, the predominant AP endonuclease is Ape1. We have employed biochemical and structural approaches to elucidate the molecular detail of the Ape1-DNA complex. Computer modeling techniques have been used to elucidate which DNA elements influence Ape1 repair activity. Insights from these studies will now allow us to design inhibitor substrates that could prove useful in anti-cancer treatment schemes. We are also developing methods to isolate dominant-negative and increased repair capacity Ape1 proteins that may serve as radio-sensitizing or -protective agents when used with gene therapy approaches. In addition, we are working to generate mammalian cell lines deleted in *APE1*. The complementary nature of the biochemical and genetic projects will permit a thorough dissection of the biological contributions of Ape1. Finally, we have identified a second human protein with similarity to Ape1, termed Ape2, which will serve as a target for future investigations.

Free radicals are generated during normal oxygen metabolism or from exposure to ionizing radiation, and attack chromosomal DNA to form oxidative damage, including 3'-blocking termini. The repair of 3'-damages is not well understood. We are designing assays to identify repair factors involved in removing obstructive 3'-ends and are presently testing several candidate 3' to 5' exonucleases. Developed *in vitro* assays may

also serve as a means for assessing individual repair capacity and for predicting one's sensitivity to ionizing radiation.

The process most involved in preventing the harmful effects of spontaneous, oxidative and alkylation DNA damage is Base Excision Repair (BER). A reduction in BER would therefore lead to an increased risk of developing disease. We have identified variation among individuals in the human population in proteins of BER (Ape1, Polymerase β, Xrcc1 and Ligase 3), and have shown that 6 of 9 variants in Ape1 lead to reduced repair activity. We are now employing biochemical assays (e.g. DNA binding and protein-protein interaction) to determine the impact of the observed variation on the integrated nature of BER. When combined with X-ray crystallography and computer molecular dynamics, these studies will determine which population-observed BER variation imparts reduced repair capacity, will elucidate the mechanistic basis for the reduced activities, and will establish a foundation for defining the relationship of genetic differences in BER to human disease susceptibility.

Nucleases are central contributors to DNA repair, recombination, and replication. Defects in these processes have been associated with increased cancer risk, neurological disorders, and premature aging. We have identified human (***HEX1/hEXO1***) and mouse (***mExo1***) genes that encode factors with homology to the *RAD2* family of nucleases. Yeast genetic studies, mammalian expression analysis, DNA substrate specificity and protein-protein interactions suggest that the mammalian Exo1 proteins function in recombination and mismatch repair. Since we have only a fundamental understanding of the biological roles of the mammalian Exo1 proteins, we are expanding our biochemical studies and expression analysis, and have established a collaboration to generate a null mouse. These investigations will help determine the functional contribution of this important mammalian nuclease and its relationship to human disease.

Publications During 3 Years of LDRD Support

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NIH R01. PI. DNA repair activities of human abasic endonuclease. Time Period: Apr. 1, 1998 to Mar. 31, 2002. Support: $1,226,751 (total) for 4 years.

NIH R01. Co-PI with Dr. Michael Thelen. Function of the human XRCC1 protein in DNA repair and recombination. Time Period: Dec. 1, 1997 to Nov. 30, 2000. Support: $1,394,132 for 4 years.

Co-investigator on five DOE projects within the Program.